macrophage-CSF (M-CSF), angiopoetin-1 (Angl) or nitric oxide synthase (NOS); or a fragment thereof.

C5 cont.

24. (Amended) A method for preventing or reducing the severity of blood vessel damage in a mammal having chronic or acute ischemia, wherein the method comprises administering to the mammal an effective amount of granulocyte macrophage-colony stimulating factor (GM-CSF); and exposing the mammal having the chronic or acute ischemia to conditions conducive to damaging the blood vessels, the amount of GM-CSF being sufficient to prevent or reduce the severity of the blood vessel damage in the mammal.

C6

48. (Amended) A method for enhancing endothelial progenitor cell (EPC) mobilization in a mammal having chronic or acute ischemia, wherein the method comprises administering an effective amount of at least one hematopoietic factor sufficient to enhance the EPC mobilization in the mammal having the chronic or acute ischemia.

Please cancel claims 4 and 16 without prejudice.

REMARKS

As an initial matter, Applicants gratefully acknowledge that claims 25-33 have been found free of the prior art. See page 29 of the Office Action.

The Declaration was objected to on grounds that it was not signed. See pg. 2 of the Action. The objection is addressed by the attached substitute Declaration that has been executed and dated by the inventors.

At pg. 2 of the Action, claims 21 and 23 were objected to on various informalities. The objection has been addressed by this submission.

The specification was objected to because it includes an embeddeed hyperlink and/or other form of browser-executable code. See pg. 2 of the Action. The objection has been addressed by this submission.

Claims 1-31 and 48-49 stand rejected under 35 USC §112, first paragraph, on various grounds. It was acknowledged in the rejection that the specification enables methods for forming new blood vessels in a mammal **having chronic or acute ischemia.** See pg 3 of the Action. Although Applicants disagree with the position taken insofar as it limits the claimed methods to the stated conditions, the rejection has been addressed by this submission. In particular, claim 1 has been amended to feature a mammal that has chronic or acute ischemia.

On pgs. 7-10 of the Action, it was alleged that the claimed methods are "highly unpredictable and immature." Applicants respectfully disagree as follows.

As an initial matter, it is requested that Dang et al. (Clin. Cancer Res. 5: 471 (1999)) be withdrawn as a reference. As cited, the publication date is well after the filing date of this application.

Even if the Dang reference is considered however, the position taken does not withstand scrutiny.

As the specification makes clear, it is possible to use DNA vascularization agents to achieve new blood vessel growth. One working in this particular field would understand from the specification that only modest expression from such agents is needed to assist that growth.

In contrast, the cited Dang, Miller & Vile, Verma & Somia, Eck& Wilson, and Deonarain references relate to a different concern ie., achieving high and long term expression from DNA constructs. Particular DNA constructs, as cited, are targetable and pose minimal immunological risk. The references however are not relevant to the invention because such DNA constructs are not needed to practice the invention. What is useful, and is provided by the instant disclosure, are DNA vascularization agents that achieve enough low expression to enhance blood vessel growth.

Successful administration and expression of a variety of DNA-based agents in vivo has been reported. Applicants have submitted under separate cover an Information Disclosure Statement (IDS) that provides some of these references.

For example, Flegner, PL and Rhodes, G (AG) summarizes work showing successful expression after direct gene transfer *in vivo*.

Further, Wolff, JA (AC) reports good gene transfer into mouse muscle *in vivo*; Takeshita, S. (AD) discloses increased expression of after direct gene transfer into skeletal muscles after injury to rats; Stratford-Perricaudet, LD. (AE) teaches widespread and long-term gene transfer to heart and other muscle tissue; Tsurumi Y. (AB) reports gene transfer of DNA encoding vascular endothelial growth factor (VEGF) and that it promotes good tissue development; and Vitadello, M. et al. (AF) discloses successful gene transfer in regenerating muscle. These and the other cited references report success with delivery and expression of a large variety DNA sequences.

Additionally, there has been therapuetic angiogenesis following gene transfer of vascular endothelian growth factor (VEGF). See Isner, JM. (AA); Tsurumi, Y. (AB) and references cited therein.

See also U.S. Pat. Nos: 5,656,465; 5,585,254, and 5,681,744 (disclosing a range of gene delivery methods).

In the face of such successful use of DNA agents, the claimed methods are simply not "unpredictable and immature" as alleged in the rejection. The references cited by the PTO cannot be allowed to foreclose Applicants from obtaining patent protection simply because a few authors opined that it was hard to express DNA under circumstances unrelated to the invention at hand. There is more than abundant evidence that workers have been routinely expressing DNA agents *in vivo*, nothwithstanding the art cited. Reconsideration and withdrawal of the rejection are thus respectfully requested.

On pgs. 10-11 of the Action, the position was advanced that the specification does not enable fragments of a vascularization modulation agent. Applicants respectfully disagree.

For example, and as the specification makes clear, practice of the invention is not limited to a particular hemoopoietic protein or angiogenic protein. According to the specification, an "effective fragment" of a vascularization modulation agent (such as GM-CSF), a hemopoietic protein, or angiogenic protein means a specific amino acid sequence that has at least 70%, preferably between from about 75% to 95% of the vessel promoting activity of the corresponding full-length protein as determined by at least one standard assay taught in the specification. See pg. 26, lines 17-29, for instance.

As an illustration, the specification provides for a suitably effective fragment of GM-CSF as having at least 70% and preferably from about 75% to 95% of the vessel promoting activity of full-length human GM-CSF. Specification at pg. 26, lines 17-29.

As understood, the rejection is premised on the notion that notwithstanding Applicants' disclosure of effective fragments of vascularization modulation agents appropriate for use with the claimed invention, use of anything but full-length agents is not enabled on grounds that it would require undue experimentation to make and use such fragments. Applicants respectfully disagree.

The specification provides examples of suitable vascularization modulation agents for use with the claimed invention including, but not limited to, full-length constructs. Should use of a particular agent fragment be needed in a specific invention embodiment, the specification provides more than ample guidance about selecting an appropriate fragment or derivative.

For example, preferred effective fragments including full-length agents exhibit good activity in at least one of the assays provided. Such assays include, but are not limited to, the standard EPC isolation assay, the standard hindlimb ischemia assay, the standard blood vessel

length assay, and the standard cornea micropocket assay. See pgs. 24-25 of the specification, for example.

Moreover, the chemical structure of many vascularization modulation agents according to the invention has been disclosed both at the amino acid and nucleic acid levels. Important function domains in the structure are recognized. Methods for producing or obtaining suitable vascularization modulation agents from commercial or public sources are also taught. See pg. 21, line 13 to pg. 23, line 5, for example.

Accordingly, it is believed that any testing needed to identify or confirm effective fragments of vascularization modulation agents for use with the invention is well within the level of experimentation permitted by the Federal Circuit. *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988).

Applicants disagree with the rejection on other grounds.

For example, a worker in this field would be able to use the guidance provided by the instant disclosure to select appropriately effective fragments of the vascularization modulation agents. The disclosure provides several assay to select such fragments. Any inoperable embodiments of the type described by the rejection could be readily avoided. As described by the Court of Customs and Appeals:

> [M]any patented claims read on vast numbers of inoperative embodiments in the trivial sense that they can and do omit 'factors which must be presumed to be within the level of ordinary skill in the art.' ... There is nothing wrong with this so long as it would be obvious to one of skill in the art how to include these factors in such manner as to make the embodiment operative rather than inoperative. *In re Cook and Merigold*, 169 USPQ 299, 302 (C.C.P.A. 1971) (quoting *In re Skrivan*, 166 USPQ 85, 88 (C.C.P.A. 1970)).

Thus, one of skill having read Applicants' disclosure would know to identify suitably effective fragments of the vascularization modulation agents in addition to more full-length constructs. Even if one assumes, *arguendo*, that a particular fragment of the agent did not

exhibit acceptable activity in an assay provided in the specification, that result, by itself, would not support the present enablement rejection. The worker would understand that another fragment as provided by the specification, could be tested and identified for suitable activity. The rejection has not provided any reason to doubt that the guidance provided by Applicants' disclosure could not be used to identify a range of effective fragments of the agents for use with the claimed methods.

Applicants respectfully disagree with the rejection on further grounds.

In particular, the J.A. Parsons reference cited on pg. 11 of the action is about a quarter of a century old. It pre-dates many advances in computer and recombinant DNA technology that were routine at the time the instant application was filed. For instance, many of the vascularization modulation agents disclosed on pg. 21, lines 13-25 have biological motifs that are readily detected. Accordingly, the "painstaking experimental study" advocated by Parsons to understand proteins is out of date. Withdrawal of the reference as relevant prior art is requested.

Concerns raised on pgs. 11-12, bridging paragraph, have been addressed by this submission.

Claims 25-31 stand rejected on grounds set forth on pgs. 12-13, bridging paragraph. Although Applicants respectfully disagree with the position taken, grounds for it have been addressed. Specifically, claim 25 has been amended to feature mammals that have chronic or acute ischemia. Reconsideration and withdrawal of the rejection are thus requested.

Claims 3, 4, 14 and 15 stand rejected under 35 USC §112, second paragraph, on various grounds. Action at pg. 14. Applicants respectfully disagree for the following reasons.

Claim 4 has been canceled. Applicants address the rejection as it now pertains to amended claim 1.

The position is taken that claims 3 and 4 are unclear for reciting the phrase "increase frequency of endothelial progenitor cells (EPC)". Respectfully, the phrase is abundantly clear particularly to one working in this area who has read the specification. For example, and as provided by the specification, an increase in EPC frequency means EPC enrichment as determined by a variety of assays disclosed by the specification. The standard EPC isolation assay is a preferred means of detecting and quantifying an increase in EPC frequency. See eg., pg. 6, lines 7-15. See also pg. 24, lines 5-16 (generally disclosing how to practice the EPC isolation assay).

See also the Drawings, especially Figures 3A-C and the accompanying figure legend on pg. 13, lines 1-3 (disclosing graphs that show increases in EPC frequency). See also the Examples section, particularly Examples 3-4 (illustrating EPC increased frequency).

Accordingly, it is submitted that the phrase "increase frequency of endothelial progenitor cells (EPC)" has clear and unambigous meaning in light of the claims and specification. Reconsideration and withdrawal of the rejection are requested.

It was also alleged that claims 14 and 15 are unclear for reciting "foci". Applicants must respectfully disagree.

As understood, "foci" is a term of art that is used by those working in this field. It references sites of neovascularization. The Drawings provide pictures of such foci. See, for instance, Figures 4A-J (providing for foci in a variety of *in vivo* and *in vitro* settings). See also Example 3 (discussing Figures 4A-J in more detail).

Accordingly, Applicants' use of the term "foci" is not unclear or ambigous. Such foci, as provided by the specification, initiate new blood vessel growth. A worker in this field would readily understand what the word foci means.

Applicants also disagree that the phrase "EPC bone marrow derived EPC" is unclear. However in the interest of furthering prosecution, the phrase has been removed from claims 14 and 15.

Claims 1, 2, 16-20, and 21-23 were rejected on various grounds under 35 USC §102. Although Applicants respectfully disagree with the positions taken, grounds for them have been addressed by this submission.

In the interest of brevity, the §102 rejections in view of the Pu et al.,. Franco or Kawakami et al, Giordano et al., Takeshita et al, or Tsurumi et al. references are considered together as follows.

Claim 1 has been amended to include language from claim 4 (now canceled). As cited, **none of the references disclose** a method for inducing formation of new blood vessels in **a mammal having acute or chronic ischemia that involves increasing frequency of EPCs by at least about 20% as determined by the standard EPC isolation assay**. In contrast, the claimed invention is based on the discovery that hematopoietic factors can be used to increase EPC mobilization (frequency) and neoascularization. See the specification at pg. 5, line 21 to pg. 6, line 15, for example. Reconsideration and withdrawal of the anticipation rejections are requested.

Claim 48 stands rejected as anticipated over the Socinski et al or Hammond et al. reference. Although Applicants respectfully disagree with the positions taken, grounds for them have been addressed.

Specifically, amended claim 48 features a method for enhancing EPC mobilization in a mammal having acute or chronic ischemia. As cited, neither the Socinski et al. or the Hammond et al. reference provides for such a method. Reconsideration and withdrawal of the rejection are requested.

In view thereof, it is submitted that all §102 rejections have been addressed.

Applicants' representative has reviewed the Grant reference cited at pg. 19 of the Action. It is not believed to the invention as claimed.

Claims 1, 3-6, 11, 12 and 14-15 stand rejected as obvious in view of the Hammond et al. (US Pat. No. 5,880,090) and Asahara et al. references. Applicants respectfully traverse the rejection as follows.

There is no express teaching, suggestion or motivation to combine the Hammond and Asahara references as done in the rejection.

For example, and as Applicants' specification makes clear, there was recognition that EPC number and/or viability decreased overtime in patients and that responsiveness to administration of angiogenic agents was limited. See pgs. 3-4 of Applicants' disclosure, bridging paragraph. The Asahara reference amplifies this point:

> A **potentially limiting factor** in strategies designed to promote neovascularization of ischemic tissues is the resident population of ECs that is competent to respond to administered cytokines (cites omitted).
>
> Asahara at pg. 967, col. 1

Thus according to Asahara and Applicants' disclosure, there was substantial doubt as to whether it was possible to promote new blood vessel growth by administering EPCs. Specifically, the cells were thought to have problems responding to cytokines (eg., Hammond's GM-CSF) to promote neovascularization. The Hammond patent, as cited, does nothing to resolve this conflict: a worker in the field would be dissuaded from administering cytokines such as GM-CSF in an attempt to engage what were viewed as potentially "unresponsive" EPCs. In marked contrast, it was Applicants who discovered that it was possible to use hematopoietic factors such as GM-CSF to increase EPC mobilization and neovascularization. See the Summary of the Invention.

On this basis alone, reconsideration and withdrawal of the rejection are requested.

Applicants disagree with the rejection on other grounds.

The cited combination of references does not provide a reasonble expectation that the claimed method would work. In particular, the cited Asahara reference cast substantial doubt as to whether neovascularization could be achieved with EPCs that were potentially limited in cytokine responsiveness. Hammond, as cited, does not remedy this shortcoming.

In view thereof, reconsideration and withdrawal of the rejection are requested.

Claims 1, 7-10 and 13 stand rejected under 35 USC §103 as being unpatentable over Bussolino or Pu et al. Action at pgs. 22-24. Claims 1 and 2 were also rejected over Bussolino at pgs. 24-25. The rejections are considered together in the interest of brevity. Although Applicants disagree with the rejections, basis for it has been addressed by this submission.

In particular, claim 1 has been amended to feature a method of inducing formation of blood vessels in a mammal that has acute or chronic ischemia by increasing endothelial progenitor cell (EPC) frequency by at least about 20% as determined by the standard EPC isolation assay. None of the cited references, taken individually or together, provide for that method.

Accordingly, reconsideration and withdrawal of the obviousness rejection are requested.

Claims 1, 21, 23 and 24 stand rejected under 35 USC §103 as being obvious over Bussolino in view of Folkman and Rosen. Although Applicants disagree with the rejections, basis for it has been addressed by this submission.

In particular, claim 1 has been amended to feature a method of inducing formation of blood vessels in a mammal that has acute or chronic ischemia by increasing endothelial progenitor cell (EPC) frequency by at least about 20% as determined by the standard EPC isolation assay. None of the cited references, taken individually or together, provide for that method.

Claims 48 and 49 stand rejected as obvious over Hammond or Socinski taken with Folkman. Although Applicants disagree with the rejection, basis for it has been addressed by this submission.

Claim 48 has been amended to recite a method for enhancing endothelial progenitor cell (EPC) mobilization in a mammal having chronic or acute ischemia. None of the cited references, taken individually or together, provide for that method.

As mentioned above, Applicants are submitting under separate cover an IDS for review by the Examiner. References not provided with the IDS can be found in co-pending application USSN 09/265,041. If the Examiner requires additional copies, the undersigned will promptly furnish them after being notified.

Applicants will further submit a Supplemental IDS form in which references considered in the co-pending application USSN 09/265,041 will be listed.

Although it is not believed that any additional fees are needed to consider this submission, the Examiner is hereby authorized to charge our deposit account no. **04-1105** should any fee be deemed necessary.

Attached to this submission is a marked-up version of the changes made to the specification and claims. The attached page is captioned "version with markings to show changes made".

Respectfully submitted,

Robert L. Buchanan (Reg. No. 40,927)
Dike, Bronstein, Roberts & Cushman
Intellectual Property Practice Group of
EDWARDS & ANGELL, LLP
P. O. Box 9169
Boston, MA 02209
Tel: (617) 439-4444
Fax: (617) 439-4170 / 7748

Date: 24 October 2002

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph on page 18, line 3-12 has been amended as follows:

Additional protein and nucleic sequences relating to the factors disclosed herein including GM-CSF can be obtained through the National Center for Biotechnology Information (NCBI)- Genetic Sequence Data Bank (Genbank). In particular, sequence listings can be obtained from Genbank at the National Library of Medicine, 38A, 8N05, Rockville Pike, Bethesda, MD 20894. Genbank is also available on the internet ~~at http://www.ncbi.nlm.nih.gov~~. See generally Benson, D.A. et al. (1997) *Nucl. Acids. Res.* 25: 1 for a description of Genbank. Protein and nucleic sequences not specifically referenced can be found in Genbank or other sources disclosed herein.

IN THE CLAIMS:

Claims 1, 13, 14, 21, 23, 24 and 48 have been amended as follows:

1. (Amended) A method for inducing formation of new blood vessels in a mammal having chronic or acute ischemia, wherein the method comprises administering to the mammal an effective amount of a vascularization modulating agent sufficient to form the new blood vessels in the mammal, and increasing endothelial progenitor cell (EPC) frequency by at least about 20% as determined by a standard EPC isolation assay.

13. (Amended) The method of claim 1, wherein the amount of administered vascularization modulating agent is sufficient to increase ~~EPC bone marrowderived~~ EPC incorporation into foci.

14. (Amended) The method of claim 13, wherein the increase in ~~EPC bone marrow derived~~ EPC incorporation into foci is at least about 20% as determined by a standard rodent bone marrow (BM) transplantation model.

21. (Amended) The method of claim 1, wherein the agent is co-administered with at least one angiogenic protein.

23. (Amended) The method of claim ~~20~~ 21, wherein the angiogenic protein is acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF-1), epidermal growth factor (EGF), transforming growth factor a and (3 (TGF-a and TFG-P), platelet-derived endothelial growth factor (PD-ECGF), platelet-derived growth factor (PDGF), tumor necrosis factor a (TNF-a), hepatocyte growth factor (HGF), insulin like growth factor (IGF), erythropoietin, colony stimulating factor (CSF), macrophage-CSF (M-CSF), angiopoetin-1 (Angl) or nitric ~~oxidesynthase~~ oxide synthase (NOS); or a fragment thereof.

24. (Amended) A method for preventing or reducing the severity of blood vessel damage in a mammal having chronic or acute ischemia, wherein the method comprises administering to the mammal an effective amount of granulocyte macrophage-colony stimulating factor (GM-CSF); and exposing the mammal having the chronic or acute ischemia to conditions conducive to damaging the blood vessels, the amount of GM-CSF being sufficient to prevent or reduce the severity of the blood vessel damage in the mammal.

48. (Amended) A method for enhancing endothelial progenitor cell (EPC) mobilization in a mammal having chronic or acute ischemia, wherein the method comprises administering an effective amount of at least one hematopoietic factor sufficient to enhance the EPC mobilization in the mammal having the chronic or acute ischemia.

Claims 4 and 16 have been cancelled, without prejudice.